KYUSHU UNIVERSITY

Public Relations Office, Kyushu University
744 Motooka Nishi-ku Fukuoka, 819-0395
TEL: +81-92-802-2443 FAX: +81-92-802-2139
E-MAIL: koho@jimu.kyushu-u.ac.jp
URL: https://www.kyushu-u.ac.jp

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Beetles under climate stress lay larger male eggs: *Wolbachia* infection drives adaptive reproduction strategy in response to rising temperature and CO_2

Researchers find that azuki bean beetles, a common pest, produce larger eggs yielding male offspring when infected with *Wolbachia* bacteria under elevated temperature and carbon dioxide conditions

Fukuoka, Japan—Researchers at Kyushu University have found that when azuki bean beetles infected with *Wolbachia* bacteria are exposed to a simulated climate change environment—characterized by elevated temperature and carbon dioxide—they tend to produce larger eggs to enhance the survivability of their offspring. Interestingly, these larger eggs gave rise exclusively to male larvae.

The study, published in *Scientific Reports*, demonstrates the benefits of *Wolbachia* infection under adverse environmental conditions in these beetles. It is also the first observation of sex-dependent changes in egg size in a species with chromosomal sex determination.

The azuki bean beetle (*Callosobruchus chinensis*) is a small insect, growing up to about 3 mm in length, and is a known pest of stored legume seeds. These beetles lay their eggs on the seed surface, and once hatched, the larvae bore into the seeds. Understanding their biology is therefore essential for mitigating the damage they cause.

"One characteristic we've observed in some insects, including these beetles, is that they increase egg size when exposed to environmental stress. Offspring hatched from larger eggs tend to survive better and develop more quickly during early stages. It's a form of biological investment under stressful conditions" explains Professor Midori Tuda of Kyushu University's Faculty of Agriculture, who led the study. "With human activities raising atmospheric CO_2 and global temperatures, it is critical to predict the future population dynamics of agricultural pests like the azuki bean beetle."

Another factor influencing egg size in insects is infection by *Wolbachia*, one of the most common parasitic microbes in insects. It can cause a variety of effects across species. For example, in thrips and mites—where sex is determined by haplodiploidy (*i.e.*, whether the offspring has a full or half set of chromosomes)—*Wolbachia*-infected parents have been shown to produce larger eggs, but only those that yield female offspring.

"We combined these two ideas to examine whether *Wolbachia* infection influences egg size under elevated temperature and CO_2 conditions, and whether this affects offspring sex," continues Tuda. "In azuki bean beetles, sex is determined by X and Y sex chromosomes, just like in humans. To our knowledge, no one has previously investigated sex-specific changes in egg size under such conditions in organisms with this type of sex determination."

Approximately 96% of natural azuki bean beetle populations are coinfected with two strains of *Wolbachia*: *wBruCon* (referred to as Con) and *wBruOri* (referred to as Ori). Single infections with either strain are rare: 1.6% of the population is infected only with Con, and 2.4% only with Ori.

The researchers exposed beetles to elevated temperature and CO_2 (denoted as eT&CO_2) for over two days during egg-laying. They found that beetles coinfected with both *Wolbachia* strains produced larger eggs, and all the larger eggs developed into male larvae. Egg size increases were

observed only in beetles coinfected with both strains of *Wolbachia*.

Interestingly, the adult lifespan of the offspring was influenced not by egg size, but by environmental conditions, sex, *Wolbachia* infection, and development time. In fact, eT&CO_2 reduced male lifespan but had no significant effect on females.

This study is the first to demonstrate sex-specific egg size changes due to *Wolbachia* infection in a species with chromosomal sex determination. Further research is needed to elucidate the mechanisms driving this sex-dependent change aided by *Wolbachia* coinfection.

"From a pest control perspective, targeting *Wolbachia* may be a strategy worth exploring. However, because nearly half of all insect species are infected with *Wolbachia*, non-selective use of bactericides could threaten non-pest insects as well," concludes Tuda. "A nature-positive approach is essential as we adapt to a changing climate."

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For more information about this research, see "*Wolbachia* infection facilitates adaptive increase in male egg size in response to environmental changes," Eloïse Leroy, Siyi Gao, Maya Gonzalez, Marie-Pierre Ellies-Oury, and Midori Tuda, *Scientific Reports* https://doi.org/10.1038/s41598-025-96680-6

About Kyushu University

Founded in 1911, Kyushu University is one of Japan's leading research-oriented institutes of higher education, consistently ranking as one of the top ten Japanese universities in the Times Higher Education World University Rankings and the QS World Rankings. The university is one of the seven national universities in Japan, located in Fukuoka, on the island of Kyushu—the most southwestern of Japan's four main islands with a population and land size slightly larger than Belgium. Kyushu U's multiple campuses—home to around 19,000 students and 8000 faculty and staff—are located around Fukuoka City, a coastal metropolis that is frequently ranked among the world's most livable cities and historically known as Japan's gateway to Asia. Through its VISION 2030, Kyushu U will "drive social change with integrative knowledge." By fusing the spectrum of knowledge, from the humanities and arts to engineering and medical sciences, Kyushu U will strengthen its research in the key areas of decarbonization, medicine and health, and environment and food, to tackle society's most pressing issues.

Fig. 1. The azuki bean beetle (*Callosobruchus chinensis*). An azuki bean beetle on an azuki bean. The white spots on either side of the bean are beetle eggs.. (Midori Tuda/Kyushu University)

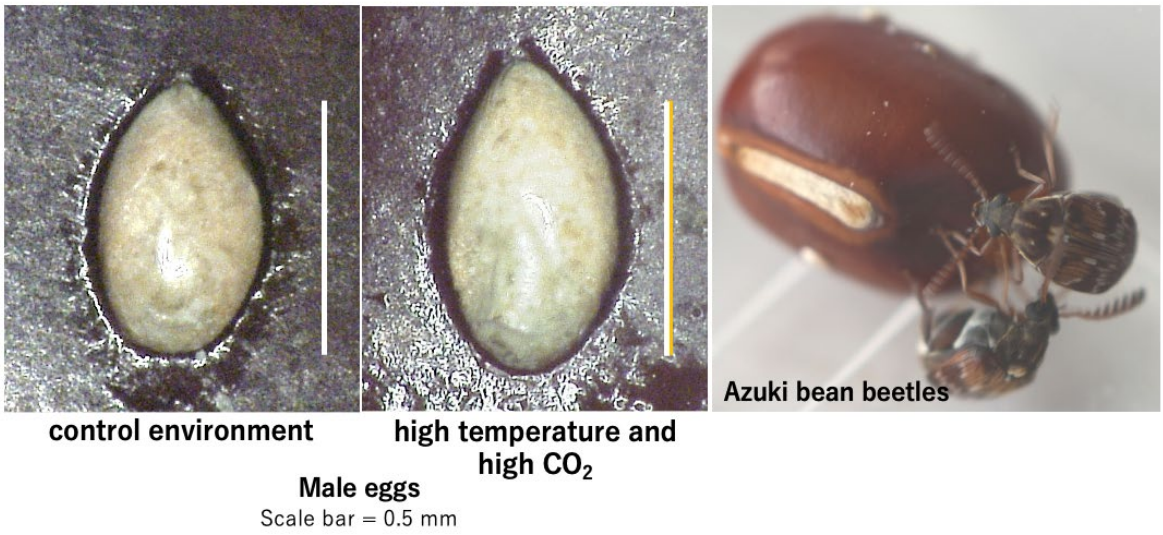

Fig. 2. Azuki bean beetles and variation in egg size across environmental conditions. Under high temperature and CO2 conditions azuki bean beetles produce larger eggs (center photo) compared to those under controlled conditions (left photo). Scale bar 0.5 mm. The photo on the right show azuki bean beetle parents on an azuki bean. (Midori Tuda/Kyushu University)

[Contact]
Midori Tuda Professor,
Department of Bioresource Sciences, Faculty of Agriculture
Tel: +81 92-802-4580
E-mail: tuda.midori.022@m.kyushu-u.ac.jp